

## REMARKS

Claims 1-15, 21, 29-30, and 55-64 are pending. Claims 16-20 and 31-54 were previously canceled. Claims 1, 21, 29, 30, 57, 59, 61, and 63 are amended. Reconsideration of the application in view of the above amendments and the following remarks is respectfully requested.

### **I. 35 U.S.C. § 112 CLAIM REJECTION (ENABLEMENT)**

**Claims 1-15, 21, 29, 30, and 55-64 comply with the enablement requirement.**

The Office maintains its rejection of Claims 1-15, 21, 29, 30, and 55-64 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. Specifically, the Office questions whether or not the specification has shown ISS, ISE, ESS, ESE, or polynucleotide sequences capable of binding human hnRNP A1 protein would work in cells other than avian cells that express hnRNP A1 protein.

Under current patent law what is claimed must be shown by Example in the patent application specification. The specification must provide evidence of how the sequences are modifying hnRNP proteins. The specification demonstrates the effect in chickens in Example 1, but it must also describe the phenotypic effect that demonstrates the interaction between the sequence and hnRNP in each system that is claimed. The Office concedes that the effect in avian cells has been enabled or “taught” in Example 1. The Office also admits that the specification teaches the sequences capable of binding to human

hnRNP A1 protein. The first issue is that the Office asserts that the specification does not teach the phenotypic effect one would look for in humans, or if the avian sequences would work in humans. The Office also asserts that the specification does not teach whether the introduction of the chicken ESS located in exon 8 into HIV infected human T cells would result in modifying hnRNP A protein within human cells. As described in the specification, the human fibroblast growth factor receptor 2 gene (*fgfr2*) contains an exon that has sequences acting as an exonic splice silencer (ESS) (Del Gatto, 1995; Del Gatto, 1996; Del Gatto-Konczak et al., 1999; Gallego et al., 1996; Graham et al., 1992; Si, 1997). The proof-of-principle experiments presented in the specification using chicken *fgfr2* exon 8 as a competitive inhibitor of chicken hnRNP A1, showing the result of fused cartilaginous skeletal elements in the limbs and *in vitro*, were based on experiments in the literature reporting (i) the published teaching that the exon-intron structure of the chicken *fgfr2* gene in the immunoglobulin domain (i.e., ligand-binding) region is essentially identical to that in the human (Johnson, D. E., and Williams, L. T. (1993)) and that structural and functional diversity in the FGF receptor multigene family exists. (Adv Cancer Res 60, 1-41), (ii) that the tissue-specific patterns of alternative splicing of *fgfr2* transcript in the human (Johnson, D. E., and Williams, L. T., 1993) and chicken (Patstone, G., Pasquale, E. B., and Maher, P. A., 1993) are highly similar; (iii) that inclusion of exon 8 rather than exon 9 in human limb mesenchymal cell *fgfr2* mRNA leads to fused skeletal elements (Oldridge, M., Zackai, E. H., McDonald-McGinn, D. M., Iseki, S., Morriss-Kay, G. M., Twigg, S. R., Johnson, D., Wall, S. A., Jiang, W., Theda,

C., Jabs, E. W., and Wilkie, A. O. (1999)) and de novo alu-element insertions in FGFR2 identify a distinct pathological basis for Apert syndrome. (Am J Hum Genet 64, 446-61); (iv) that human hnRNP A1 deficiency causes this missplicing in the human fgfr2 gene (Del Gatto-Konczak et al., 1999); and (v) that it does so by interacting with exon 8 in human fgfr2 pre-mRNA (Del Gatto-Konczak et al., 1999),. Since our proof-of-principle interference with chicken fgfr2 alternative splicing using chicken fgfr2 exon 8 as a competitor for chicken hnRNP A1 was motivated almost entirely by human *in vitro* and clinical data, and the results of the proof-of-principle experiment were to reproduce a specific human skeletal dysmorphology (Apert syndrome, see Oldridge, 1999) in which skeletal elements are fused by causing fusion in chicken limbs in ovo (see example 1), it is entirely expected that if a similar experiment were done in human embryos (which is ethically proscribed), it would lead experimentally to similar skeletal fusions as were found in the chicken embryos (and are found naturally in humans with Apert syndrome). The predicted transfer of this technology from the chicken proof-of-principle demonstration to the human situation (hypothetical in this case) is also based on the fact that the known polyribonucleotide target for human hnRNP A1 in exon 8 of the human fgfr2 transcript, designated S10, has the sequence (5'-UAGGGCAGGC-3') (Del Gatto-Konczak et. al. 1999). The chicken fgfr2 transcript, which the specification teaches has an analogous binding site for chicken hnRNP A1, differs from S10 by only one base, having the sequence 5'-UAGGGAGGGC-3' (GenelD: 396259).

With respect to phenotypic effects of potential therapeutic uses of the invention, the specification indicated that the *tat* gene of the human immunodeficiency virus type 1 (HIV-1) also has an ESS that interacts with hnRNP A1 (Amendt et al., 1994; Amendt et al., 1995; Caputi et al., 1999). The life cycle of HIV-1 requires alternative splicing (Amendt, B.A., Si, Z.H., and Stoltzfus, C.M., 1995; Si, Z., Amendt, B.A., and Stoltzfus, C.M., 1997; Si, Z. H., Rauch, D., and Stoltzfus, C.M., 1998; Del Gatto-Konczak, F., Olive, M., Gesnel, M.C., and Breathnach, R., 1999). The reason for this is that HIV is a retrovirus: its genome consists of RNA which must be transmitted to the next generation of virus intact (i.e., unspliced), but the RNA serves as a messenger RNA, not only a genetic template, and a fraction of the RNA molecules must get spliced if the appropriate complement of proteins, such as *tat*, are to be synthesized.

In the HIV life cycle, therefore, splicing must occur, but it must be inefficient to provide a pool of unspliced messages which encode viral proteins and serve as genomes for new virions (Caputi, M., Mayeda, A., Krainer, A.R., and Zahler, A.M., 1999). Indeed, virus production is arrested in a natural HIV variant that has an aberrant ESS (Wentz, M.P., Moore, B.E., Cloyd, M.W., Berget, S.M., and Donehower, L.A., 1997). At the time the specification was filed it was already known (and cited therein) that HIV employed the human host's endogenous hnRNP A1 to block splicing by acting on the *tat* ESS (Caputi, M., Mayeda, A., Krainer, A. R., and Zahler, A. M. (1999). hnRNP A/B proteins are required for inhibition of HIV-1 pre-mRNA splicing. EMBO J 18, 4060-7; Del Gatto-Konczak et. al. 1999).

If a polynucleotide or synthetic analogue containing the HIV *tat* ESS, which thereby competed with the alternative splicing regulatory protein hnRNP A1 were introduced into HIV infected cells the inevitable consequence is that the viral infection would be attenuated, and with it, presumably viremia-related symptoms in the infected host. Identifying the relevant competitor polynucleotide would not take undue experimentation—it is already known: in particular, the sequence of the HIV *tat* gene was well-known at the time that the specification was filed, and the sequence of exon 2 of this gene, the splicing of which is blocked by the action of hnRNP A1, was also known (Del Gatto-Konczak et. al. 1999).

The second issue raised by the office is showing or “teaching” how to ensure that the introduced sequences will bind to hnRNP A and not be saturated out by the presence of other binding proteins, such as SF2/ASF. The Office is looking for a particular sequence, or other methodology or experimental protocol that demonstrates that at least one of the introduced sequences will interact with hnRNP A and not be quenched by other binding proteins, or cause the phenotypic effect independent of binding to hnRNP A. The Office is asking for a particular sequence that only hnRNP A will bind to, or a protocol that ensures the introduced sequence will bind to hnRNP A in each embodiment we would like to claim. The Office admits that the art teaches ISS, ISE, ESS, ESE, or polynucleotide sequences capable of binding human hnRNP A1 protein.

In the proof-of-principle demonstration depicted in Fig. 5 of the Specification, panels 5a and 5b show respectively the *in vitro* and *in vivo*

phenotypes obtained when the polynucleotide sequence corresponding to exon 8 of chicken *fgfr2* is transfected into the developing chicken limb. These phenotypes show fusion of skeletal structures similar to that of certain severe cases of Apert syndrome in humans which are associated with the aberrant use of exon 8 in the *fgfr2* transcript rather than the exon 9 normally used in *fgfr2* transcripts in limb skeletogenic tissues (Oldridge, 1999). Since the action of hnRNP A1 is precisely what causes exon 8 not to be incorporated into the *fgfr2* transcript in tissues where exon 9 is used (Del Gatto-Konczak et. al. 1999), the phenotypes of Figs. 5a and b were the expected ones if our transfected polynucleotide (i.e., *fgfr2* exon 8) was working as a splice choice antagonist. In order to confirm that the results in Figs. 5a and 5b were indeed associated with misincorporation of exon 8 into the transcript, we performed the northern blot shown in Fig. 5c of the Specification. As described there, limb cells transfected with *fgfr2* exon 8 (138 bases of single-stranded RNA, containing an ESS recognized by hnRNP A1) exhibited full length *fgfr2* mRNA with exon 8 incorporated, which is uncharacteristic for this tissue. The specificity of this intervention with regard to RNA sequence was shown in the same Northern blot of Fig. 5c, in which cells transfected with *fgfr2* exon 9 (141 bases of single stranded RNA, not containing an ESS recognized by hnRNP A1) had no effect on the exon composition of the *fgfr2* transcript, leaving it with the normal inclusion of exon 9. This also corresponds to the lack of "skeletal fusion" phenotype in the exon 9-transfected culture in Fig. 5a. With the exon 9 controls, the result of the exon 8 transfection experiments *in vitro* and *in vivo* have no

reasonable explanation other than the specific blocking of hnRNP A1 by the polynucleotide containing the ESS to which it normally binds.

The use of a complete exon in the proof-of-principle demonstration was facilitated by the fact that exon 8 of fgfr2 contains an exonic splicing silencer but not also an exonic splicing enhancer. If it contained both, then the in trans use of the full exon would compete with both the suppression and enhancement of splicing, and not have a clear-cut effect.

Applicant has amended Claims 1-15, 21, 29, 30, and 55-64 to comply with the enablement requirement. Claims 1-15, 21, 29, 30, and 55-64 as amended add the phrase "human or avian." The claims have therefore been amended to overcome this rejection by limiting the claims to modifying either human or avian hnRNP A proteins, both of which the specification teaches. This rejection is respectfully traversed. Applicant respectfully requests the withdrawal of this rejection.

## **II. 35 U.S.C. § 102 CLAIM REJECTION**

**Claims 1, 3, 14, 15, 21, 29, 30, 55-58, and 61-64 are patentably distinguishable from Purcell and Martin.**

The Office rejected Claims 1, 3, 14, 15, 21, 29, 30, 55-58, and 61-64 under 35 U.S.C. § 102(b) as being anticipated by Purcell and Martin. This rejection is respectfully traversed.

The Office asserts that the procedure of infecting lymphocytes or human T-cell lines with HIV-1 virus is disclosed in Purcell and Marin, and since the HIV

genome contains ESS, ISS, and ESE sequences that hnRNP A proteins bind to, our method is anticipated or already known in the art.

Claims 1, 29, 57, 61, and 63 have been amended to include the phrase “consisting essentially of” to distinguish the subject matter of these claims from Purcell and Martin, which include the HIV genome. Purcell and Martin studied infecting lymphocytes or human T-cell lines with HIV-1 virus by contacting the cell lines with HIV-1 virus. Because Applicant uses RNA that does not include the HIV genome, Applicants’ use of RNA polynucleotide sequences are distinguishable from Purcell and Martin’s HIV genome.

Further, viral transfections, such as HIV transfection, have known phenotypic effects on cells, but these effects are due to the virus’s ability to replicate and specify RNA and proteins. The piece of RNA introduced into cells as per the Specification would contain one or more of the ESSs contained the 267 bp *tat* exon 2 (Del Gatto-Konczak et. al. 1999) and not the ESE also contained in that exon. (Alternatively, the ESE alone could be used as a splice choice antagonist in HIV therapy, since too little splicing, like too much splicing, would also be lethal to the viral life cycle). These molecules have no ability to act autonomously, since they are not transcription- or replication-competent. While the exon is part of the HIV-1 genome, the function of portions of it to act as *in cis* to suppress splicing of some viral genomes i.e., those that will be packaged and propagated to the next generation of virus, this is exactly what is antagonized by the introduction of competitive polynucleotide splice choice antagonists acting in trans. (Similarly, ESE antagonists acting in trans will suppress splicing and

prevent viral proteins from being made). Nothing in the scientific literature teaches that fragments of a viral genome acting in trans to compete with essential proteins of the viral life cycle is a mechanism that is employed in the natural system. Thus, Applicants' invention is patentably distinguishable from Purcell and Martin. Applicant respectfully requests the withdrawal of this rejection.

### **III. 35 U.S.C. § 112 CLAIM REJECTION (WRITTEN DESCRIPTION)**

**Claims 1-15, 21, 29, 30, 55, and 56 comply with the written description requirement.**

Claims 1-15, 21, 29, 30, 55, and 56 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Office asserts that the claims contain subject matter that was not described in the specification in a way that would reasonably convey to one of ordinary skill in the art that the inventors had possession of the claimed invention. The claims are rejected for failing to provide written description and evidence of possession of the claimed genus of RNAs (those with a sufficient homology to at least a portion of FGFR2 exon 8) being used in the claimed method. The phrase sufficient homology is deleted from the subject claims to overcome this rejection. The sequences of FGFR2 exon 8 capable of binding to the hnRNP A protein have been extensively documented (Del Gatto, 1995, Del Gatto, 1996, Chabot, 1996, Siomi and Dreyfuss, 1997, Lopez, 1998, and Del Gatto-Konczak et al., 1999). Further, the functional characteristics of these sequences has been

described. The claimed sequences must be capable of binding to hnRNP A protein. Because both the functional and physical characteristics are described in the specification, Applicants' invention meets the written description requirement. Applicant respectfully requests the withdrawal of this rejection.

## V. CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that the claims of the present invention define subject matter patentable over the references cited by the Office and that the application is in condition for allowance. Should the Office believe that anything further is desirable to place the application in better condition for allowance, the Office is invited to contact Applicants' undersigned attorney at the below listed telephone number.

The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to deposit account number 03-2469. Moreover, if the deposit account contains insufficient funds, the Commissioner is hereby invited to contact Applicant's undersigned representative to arrange payment.

Respectfully submitted,

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